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  FILE 'MEDLINE, BIOSIS, CAPLUS' ENTERED AT 16:54:58 ON 10 OCT 2003
L1        495645 S NMR
L2        229272 S L1 AND LIGAND OR BINDER
L3             3 S L2 AND AMINO ACID PAIR
                 E WEIGELT
L4            18 S E3
L5          1073 S DUAL LABELING
L6            19 S L5 AND AMINO ACIDS
L7             0 S SCREENING AMINO ACIDS PAIRS
L8            16 S SCREENING AMINO ACIDS
L9             0 S L8 AND NMR
L10           16 S SCREENING AMINO ACIDS
L11            0 S 50 TO 1000 DA
L12           80 S 50 DA
L13       495645 S NMR
L14            0 S L13 AND L12
L15           39 S BINDER MOLECULES
L16            2 S L15 AND NMR
L17            1 S L15 AND AMINO ACIDS
L18            1 S ISOTOPE DUAL LABELING
L19            0 S SCREENING BINDER MOLECULES

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> e wikstrom
E1          10      WIKSTROL/BI
E2           2      WIKSTROLS/BI
E3          94  --> WIKSTROM/BI
E4           1      WIKSTROMAL/BI
E5          44      WIKSTROMOL/BI
E6           2      WIKSTROSIN/BI
E7           4      WIKSTROTOXIN/BI
E8           2      WIKSTROTOXINS/BI
E9           7      WIKSWO/BI
E10          1      WIKT/BI
E11          1      WIKT11/BI
E12          1      WIKTELIUS/BI
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Department of Physical Chemistry, Lund University, Sweden.
SO BIOCHEMISTRY, (1987 Oct 20) 26 (21) 6723-35.
Journal code: 0370623. ISSN: 0006-2960.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198803
ED Entered STN: 19900305
Last Updated on STN: 19970203
Entered Medline: 19880307

AB Genes encoding the minor A component of bovine calbindins D9k--the smallest protein known with a pair of EF-hand calcium-binding sites--with **amino acid** substitutions and/or deletions have been synthesized and expressed in Escherichia coli and characterized with different biophysical techniques. The mutations are confined to the N-terminal Ca2+-binding site and constitute Pro-20----Gly (M1), Pro-20----Gly and Asn-21 deleted (M2), Pro-20 deleted (M3), and Tyr-13----Phe (M4). 1H, 43Ca, and 113Cd **NMR** studies show that the structural changes induced are primarily localized in the modified region, with hardly any effects on the C-terminal Ca2+-binding site. The Ca2+ exchange rate for the N-terminal site changes from 3 s-1 in the wild-type protein (M0) and M4 to 5000 s-1 in M2 and M3, whereas there is no detectable variation in the Ca2+ exchange from the C-terminal site. The macroscopic Ca2+-binding constants have been obtained from equilibration in the presence of the fluorescent chelator 2-[[2-[bis(carboxymethyl)-amino]- 5-methylphenoxy]methyl]-6-methoxy-8-[bis(carboxymethyl)amino]quinoline or by using a Ca2+-selective electrode. The Ca2+ affinity of M4 was similar to that of M0, whereas the largest differences were found for the second stoichiometric step in M2 and M3. Microcalorimetric data show that the enthalpy of Ca2+ binding is negative (-8 to -13 kJ.mol-1) for all sites except the N-terminal site in M2 and M3 (+5 kJ.mol-1). The binding entropy is strongly positive in all cases. Cooperative Ca2+ binding in M0 and M4 was established through the values of the macroscopic Ca2+-binding constants. Through the observed changes in the 1H **NMR** spectra during Ca2+ titrations we could obtain ratios between **site binding** constants in M0 and M4. These ratios in combination with the macroscopic binding constants yielded the interaction free energy between the sites delta delta G as -5.1 +/- 0.4 kJ.mol-1 (M0) and less than -3.9 kJ.mol-1 (M4). There is evidence (from 113Cd **NMR**) for site-site interactions also in M1, M2, and M3, but the magnitude of delta delta G could not be determined because of sequential Ca2+ binding.

ANSWER 6 OF 21 MEDLINE on STN

AN 89088173 MEDLINE

DN 89088173 PubMed ID: 3061464

TI Purification and **NMR** studies of [methyl-13C]methionine-labeled truncated methionyl-tRNA synthetase.

AU Rosevear P R

CS Department of Biochemistry and Molecular Biology, University of Texas Medical School, Houston 77225.

SO BIOCHEMISTRY, (1988 Oct 4) 27 (20) 7931-9.
Journal code: 0370623. ISSN: 0006-2960.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 198902

ED Entered STN: 19900308
Last Updated on STN: 19980206
Entered Medline: 19890223

dup

AB A procedure for the rapid purification of a truncated form of the Escherichia coli methionyl-tRNA synthetase has been developed. With this procedure, final yields of approximately 3 mg of truncated methionyl-tRNA synthetase per gram of cells, carrying the plasmid encoding the gene for the truncated synthetase [Barker, D.G., Ebel, J.-P., Jakes, R., & Bruton, C.J. (1982) Eur. J. Biochem. 127, 449], can be obtained. The catalytic properties of the purified truncated synthetase were found to be identical with those of the native dimeric and trypsin-modified methionyl-tRNA synthetases. A rapid procedure for obtaining milligram quantities of the enzyme is necessary before the efficient incorporation of stable isotopes into the synthetase becomes practical for physical studies. With this procedure, truncated methionyl-tRNA synthetase labeled with [methyl-13C]methionine was purified from an Escherichia coli strain auxotrophic for methionine and containing the plasmid encoding the gene for the truncated methionyl-tRNA synthetase. Both carbon-13 and proton observe-heteronuclear detect **NMR** experiments were used to observe the 13C-enriched methyl resonances of the 17 methionine residues in the truncated synthetase. In the absence of ligands, 13 of the 17 methionine residues could be resolved by carbon-13 **NMR**. Titration of the synthetase, monitoring the chemical shifts of resonances B and M (Figure 3), with a number of **amino acid** ligands and ATP yielded dissociation constants consistent with those derived from binding and kinetic data, indicating active **site binding** of the ligands under the conditions of the **NMR** experiment.(ABSTRACT TRUNCATED AT 250 WORDS)

ANSWER 14 OF 21 CAPLUS COPYRIGHT 2003 ACS on STN

AN 2001:658237 CAPLUS

DN 135:354331

TI **NMR** Structure of the hRap1 Myb Motif Reveals a Canonical Three-helix Bundle Lacking the Positive Surface Charge Typical of Myb DNA-Binding Domains

AU Hanaoka, Shingo; Nagadoi, Aritaka; Yoshimura, Shoko; Aimoto, Saburo; Li, Bibo; de Lange, Titia; Nishimura, Yoshifumi

CS Graduate School of Integrated Science, Yokohama City University, Tsurumi-ku, Yokohama, 230-0045, Japan

SO Journal of Molecular Biology (2001), 312(1), 167-175
CODEN: JMOBAK; ISSN: 0022-2836

PB Academic Press

DT Journal

LA English

bad

AB Mammalian telomeres are composed of long tandem arrays of double-stranded telomeric TTAGGG repeats assocd. with the telomeric DNA-binding proteins, TRF1 and TRF2. TRF1 and TRF2 contain a similar C-terminal Myb domain that mediates sequence-specific binding to telomeric DNA. In the budding yeast, telomeric DNA is assocd. with scRap1p, which has a central DNA-binding domain that contains two structurally related Myb domains connected by a long linker, an N-terminal BRCT domain, and a C-terminal RCT domain. Recently, the human ortholog of scRap1p (hRap1) was identified and shown to contain a BRCT domain and an RCT domain similar to scRap1p. However, hRap1 contained only one recognizable Myb motif in the center of the protein. Furthermore, while scRap1p binds telomeric DNA directly, hRap1 has no DNA-binding ability. Instead, hRap1 is tethered to telomeres by TRF2. Here, we have detd. the soln. structure of the Myb domain of hRap1 by **NMR**. It contains three helixes maintained by a hydrophobic core. The architecture of the hRap1 Myb domain is very close to that of each of the Myb domains from TRF1, scRap1p and c-Myb. However, the electrostatic potential surface of the hRap1 Myb domain is distinguished from that of the other Myb domains. Each of the minimal DNA-binding domains, contg. one Myb domain in TRF1 and two Myb domains in scRap1p and c-Myb, exhibits a pos. charged broad surface that contacts closely the neg. charged backbone of DNA. By contrast, the hRap1 Myb domain shows no distinct pos. surface, explaining its lack of DNA-binding activity. The hRap1 Myb domain may be a member of a second class of Myb motifs that lacks DNA-binding activity but may interact instead with other proteins. Other possible members of this class are the c-Myb R1 Myb domain and the Myb domains of ADA2 and Adf1. Thus, while the folds of all Myb domains resemble each other closely, the function of each Myb domain depends on the **amino acid** residues that are located on the surface of each protein. (c) 2001 Academic Press.

N 136:306415
TI New **NMR** screening method
IN Wikstroem, Mats; Weigelt, Johan
PA Biovitrum AB, Swed.
SO PCT Int. Appl., 34 pp.
CODEN: PIXXD2
DT Patent
LA English

Applicant's work

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002033406	A1	20020425	WO 2001-SE2281	20011019
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	AU 2001096165	A5	20020429	AU 2001-96165	20011019
	US 2002119496	A1	20020829	US 2001-986240	20011019
	EP 1327144	A1	20030716	EP 2001-977015	20011019
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
PRAI	SE 2000-3811	A	20001020		
	US 2000-243626P	P	20001026		
	WO 2001-SE2281	W	20011019		

AB The invention refers to a method for identifying at least one binder mol. comprising the steps of: (a) choosing two amino acid types (AA1 and AA2) in a polypeptide or protein of interest, whereby AA2 at least once occurs directly subsequent to AA1 in the amino acid sequence of the polypeptide or protein, defining an amino acid pair AA1-AA2; (b) labeling the two amino acid types (AA1 and AA2) in the polypeptide or protein of interest, whereby all AA1-residues is labeled with 13C and all AA2-residues with 15N; (c) generating a first HNCO-type **NMR** spectrum of the labeled polypeptide or protein from step (b), thereby identifying signals from the labeled amino acid pair AA1-AA2; (d) contacting the labeled polypeptide or protein with a potential binder mol. or a mixt. of **binder mols**. under conditions and sufficient time for allowing binding of the potential binder mol.(s) and the labeled polypeptide or protein; (e) generating a second HNCO-type **NMR** spectrum, or a 1H-15N correlation type **NMR** spectrum, of the mix from step (d), monitoring signals identified in step (c); (f) comparing the first and the second **NMR** spectra, whereby a chem. shift change of the signals identified in step (c) between the two spectra indicates an interaction between the potential binder mol. and the labeled polypeptide or protein.

ANSWER 1 OF 21 MEDLINE on STN
AN 2002231684 MEDLINE
DN 21965998 PubMed ID: 11969409
TI Prime **site binding** inhibitors of a serine protease: NS3/4A of hepatitis C virus.
AU Ingallinella Paolo; Fattori Daniela; Altamura Sergio; Steinkuhler Christian; Koch Uwe; Cicero Daniel; Bazzo Renzo; Cortese Riccardo; Bianchi Elisabetta; Pessi Antonello
CS IRBM P. Angeletti, Via Pontina Km 30.600, 00040 Pomezia (Rome), Italy.
SO BIOCHEMISTRY, (2002 Apr 30) 41 (17) 5483-92.
Journal code: 0370623. ISSN: 0006-2960.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200205
ED Entered STN: 20020424
Last Updated on STN: 20020530
Entered Medline: 20020529
AB Serine proteases are the most studied class of proteolytic enzymes and a primary target for drug discovery. Despite the large number of inhibitors developed so far, very few make contact with the prime site of the enzyme, which constitutes an almost untapped opportunity for drug design. In the course of our studies on the serine protease NS3/4A of hepatitis C virus (HCV), we found that this enzyme is an excellent example of both the opportunities and the challenges of such design. We had previously reported on two classes of peptide inhibitors of the enzyme: (a) product inhibitors, which include the P(6)-P(1) region of the substrate and derive much of their binding energy from binding of their C-terminal carboxylate in the active site, and (b) decapeptide inhibitors, which span the S(6)-S(4)' subsites of the enzyme, whose P(2)'-P(4)' tripeptide fragment crucially contributes to potency. Here we report on further work, which combined the key binding elements of the two series and led to the development of inhibitors binding exclusively to the prime site of NS3/4A. We prepared a small combinatorial library of tripeptides, capped with a variety of constrained and unconstrained diacids. The SAR was derived from multiple analogues of the initial micromolar lead. Binding of the inhibitor(s) to the enzyme was further characterized by circular dichroism, site-directed mutagenesis, a probe displacement assay, and **NMR** to unequivocally prove that, according to our design, the bound inhibitor(s) occupies (occupy) the S' subsite and the active site of the protease. In addition, on the basis of the information collected, the tripeptide series was evolved toward reduced peptide character, reduced molecular weight, and higher potency. Beyond their interest as HCV antivirals, these compounds represent the first example of prime site inhibitors of a serine protease. We further suggest that the design of an inhibitor with an analogous binding mode may be possible for other serine proteases.